IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Daniel P. Little *et al.*

Appln. No.: 081786,988

Filed: January 23, 1997

For: SYSTEMS **AND** METHODS FOR PREPARING AND ANALYZING LOW VOLUME ANALYTE ARRAY ELEMENTS

Examiner: Yelena G. Gakh

Art Unit: 1743

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Declaration is filed concurrently with a Response to an Office action issued April 13,2004, in connection with the above-identified patent application.

1. I, Thomas Becker, hold a Diploma in Physics, and have researched and developed mass spectrometric devices for the past 10 years. I was a member of the laboratory that developed the technology disclosed in the above-identified patent application, and therefore, I am familiar with the technology and the state of the art at the time of its development. I have reviewed the claims amended in the Response filed concurrently, the above-identified patent application, the Office action dated April 13,2004 and documents cited therein.

2. The documents cited in the Office action dated April 13, 2004 pertain to mass spectrometric analysis of proteins and peptides and not analysis of nucleic acid molecules. Examples of the protein/peptide matrix components are ferulic acid, sinapic acid, alpha-cyano-3-hydroxy-cinnamic acid, and alpha-cyano-4-hydroxy-cinnamic acid. The matrix 3-hydroxypicolinic acid (3-HPA) is useful for mass spectrometric analysis of nucleic acid molecules, and is not discussed in the documents cited in the Office Action. To my knowledge, the 3-HPA matrix had been applied in **microliter** volumes to substrates in combination with analyte, **but not nanoliter** volumes without analyte. The 3-HPA matrix was known to have properties different than the protein/peptide matrix solutions, as described below.

3. The cited documents discuss methods in which far larger amounts of matrix are deposited on the substrate. For example, when comparing concentrations and volumes of matrix applied using the methods described in Nicola *et al.* and the present patent application, the smallest amount of matrix spotted in Nicola *et al.* is about 10 times larger than the largest amount of matrix spotted in the claimed subject matter. Thus, the amount of matrix pre-deposited in the claimed methods and substrates is less than the amount described in the cited documents. Also, the spot diameter for the claimed methods and substrates is far less than those described in the cited documents. For example, the spot diameter of the claimed substrates does not exceed 1.13 millimeters, whereas the spot diameter described in Nicola et al. and Vorm *et al.* is several millimeters (*e.g.*, 10 millimeters) in diameter. Accordingly, the cited documents discussed methods in which larger amounts of matrix and larger spot sizes were applied to substrates.

4. The protein/peptide matrix components typically form small, homogeneous crystals when spotted on a substrate. This small crystal homogeneity facilitates accurate spot-to-spot mass spectra reproducibility. In contrast, 3-HPA applied under the conditions reported in the documents cited by the Office yields a crystal morphology that does not provide consistent mass spectra results. Spots resulting from deposition of analyte on the 3-HPA matrix spots pre-deposited under conditions of the cited documents often have amorphous structure. These amorphous structures often lead to poor mass spectra reproducibility.

5. Research pertaining to protein/peptide matrix components was not applicable to developing the claimed substrates for mass spectrometric analysis due to the different 3-HPA crystal morphology (*i.e.*, amorphous structure). It was not until the claimed substrates were generated using the methods and equipment described in the specification (*i.e.*, pre-deposition of 3-HPA matrix in nanoliter volumes, resulting in a more crystalline and less amorphous matrix/analyte structure, upon analyte deposition), and then analyzed, that it was determined they could be successfully utilized for reproducible mass spectrometric analysis.

6. I believe the facts and statements herein are accurate and true. I understand knowingly and willfully (1) falsifying, concealing, or covering up by any trick, scheme, or device a material fact; (2) making any materially false, fictitious, or fraudulent statement or representation; or (3) making or using any false writing or document knowing the same to contain any materially false, fictitious, or fraudulent statement or entry, is punishable by fine or imprisonment or both under 18 U.S.C. §1001.

Respectfully submitted,

By: _____ Dated: 10/12/05

Thomas Becker
Principal Scientist